Joon-Haeng RHEE, et al.

Atty. Dkt.: Q95704

Preliminary Amendment

Page 2

AMENDMENTS TO THE SPECIFICATION

Please replace paragraph nos. 14 and 15 (page 3) with the following rewritten

paragraphs:

Fig.3 shows the result of completely protection of the host from lethal doses of tetanus

toxin after immunization with $1 \oplus \mu g$, $5 \oplus \mu g$ and $15 \oplus \mu g$ of FlaB mixed with the tetanus toxoid

via mice transnasal route.

Fig. 4 shows the result of the antigen specific immune response measured by the ELISA

method using sampled mice sera and various mucus samples after immunization with $1 \oplus \mu g$, 5

 $\exists \mu g$ and 15 $\exists \mu g$ of FlaB mixed with the tetanus toxoid via the mice transnasal route.

Please replace paragraph nos. 55, 56 and 57 (pages 8-9) with the following rewritten

paragraphs:

V. vulnificus MO6-24/O type strains (obtained from J. Glenn Morris, Division of

Hospital Epidemiology, University of Maryland School of Medicine, USA) and mini-Tn5 lacZ1

containing E. coli SM10\(\lambda\)pir strains (obtained from Kenneth N. Timmis, GBF National Research

Center for Biotechnology, Braunschweig, Germany) were cultured overnight at 37°C, 210 rpm in

a stirring incubator, each were inoculated with single colony at 10 ml of 2.5 HI(2.5% NaCl heart

infusion) broth media and 20ml of LB (containing 100 $\oplus \mu$ g/ml of Ampicillin and 100 $\oplus \mu$ g/ml of

Kanamicin) broth media.

The following day these were centrifuged, and washed with antibiotic-free LB broth

media and centrifuged two times, then suspended at $100 \oplus \mu\ell$ of new LB broth media. Each

Joon-Haeng RHEE, et al.

Atty. Dkt.: Q95704

Preliminary Amendment

Page 3

bacterial suspension of E. coli and V. vulnificus were mixted together and dropped on LB agar plate. After culturing it overnight at 37°C, $800 \oplus \mu \ell$ of new 2.5 HI broth media was added to the grown colonies on LB agar plate and the grown colonies was scraped carefully after using sterilized glass rods. This bacterial suspension was moved to a 1.5 ml plastic test tube and suspended until becoming homogenous state. The suspension was diluted to 1/10 and 1/100, then undiluted and the dilutes dropped on TCBS (thiosulfate citrate bile sucrose) agar plate containing 200 \(\preceq\mu\)/g/ml of Kanamycin, spread until sufficiently penetrated, and cultured overnight at 37°C.

The following day only Vibrio colonies, grown on TCBS agar plate, were taken and inoculated on TCBS agar plate containing 300 $\oplus \mu g/ml$ of Kanamycin using toothpicks, and overnight cultured at 37°C. The following day grown Vibrio colonies were inoculated on 96wells culture plates, containing 100 $\oplus \mu \ell$ of 2.5HI with 200 $\oplus \mu g/ml$ of Kanamycin, and cultured overnight at 37°C without stirring. The following day 80 $\oplus \mu \ell$ of 50% glycerol was added to each well, containing grown bacteria, and stored at -80°C in a deep freezer. When used for the experiments, these were inoculated to 2.5 HI broth media and cultured as needed.

Please replace paragraph nos. 72 and 73 (page 10) with the following rewritten paragraphs:

Seven-week-old female Balb/c mice were intranasally immunized three times with 20⊕ $\underline{u\ell}$ of PBS (phosphate buffered saline), $3 \oplus \underline{ug}$ of tetanus toxoid alone, or with combinations of 3 Joon-Haeng RHEE, et al.

Atty. Dkt.: Q95704

Preliminary Amendment

Page 4

 $\exists \mu$ g of tetanus toxoid and $1 \exists \mu$ g, $5 \exists \mu$ g and $15 \exists \mu$ g of FlaB of V. vulnificus, at 7-day intervals.

Seven days after the last immunization, saliva, vaginal wash and serum samples were collected

from the immunized mice to assess TT-specific systemic immune responses and mucosal

immune responses. These responses were measured by ELISA (Enzyme linked immuno sorbant

assay) methods, and the mice that were vaccinated 3 times before were observed for 7 days after

systemic administration of minimally 200 folds of lethal doses of tetanus toxoid. The results are

shown in Figures 3 and 4.

As shown in Fig.3, the mice of the control group immunized with PBS only - were all

dead (100%) within 24 hours, and only the 17% of the group of mice intranasally immunized

with tetanus toxoid (TT) only had survived. However 10%100% of group of mice immunized

with a combination of tetanus toxoid and $1 \oplus \mu g$, $5 \oplus \mu g$ or $15 \oplus \mu g$ of FlaB of V. vulnificus (TT

+Vv-FlaB) had survived. The survived mice of TT showed tonic paralyses, but the group of TT

+ Vv-FlaB showed the same features as normal mice.

Please replace Table 2, paragraph no. 76 (pages 10-11) with the following rewritten

paragraph:

Joon-Haeng RHEE, et al. Atty. Dkt.: Q95704 Preliminary Amendment Page 5

Table 2

Groups	Protective immunity test (against tetanus toxoid)	Survival rate (7 days)
naive(n=5)	+	0%
TT(tetanus toxoid) only (n=15)	+	17%
TT + 7⊕ <u>µg</u> Lm-FlaA (n=5)	+	100%
TT + 9 <u>□μg</u> Vv-FlaB (n=5)	+	100%
TT + 12⊕ <u>μg</u> St-FliC (n=5)	+	100%